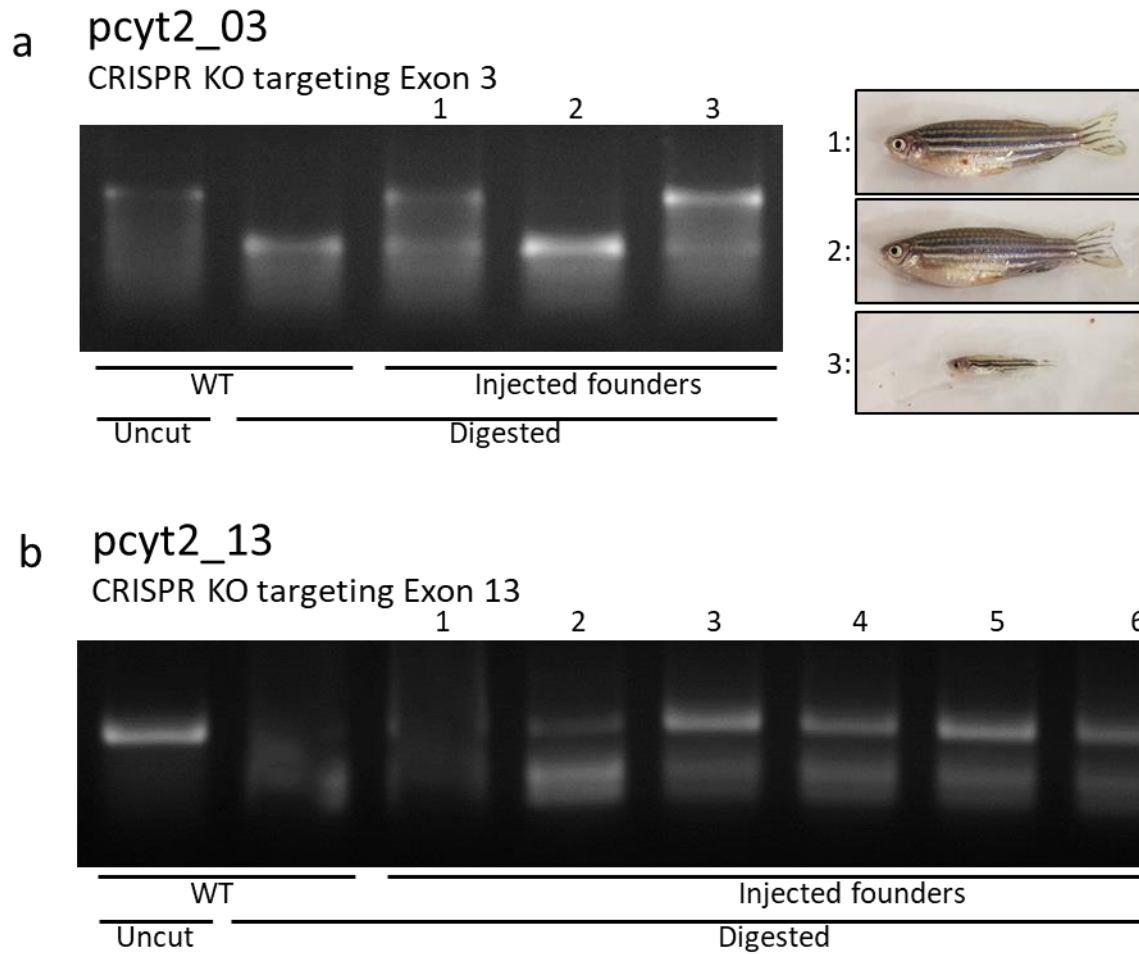
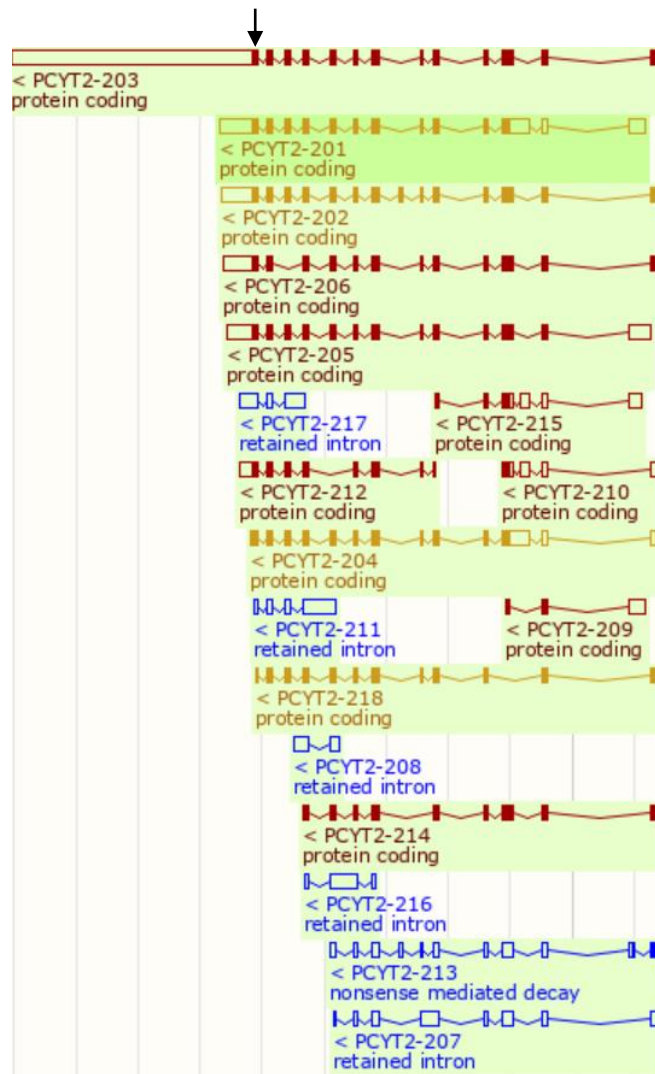


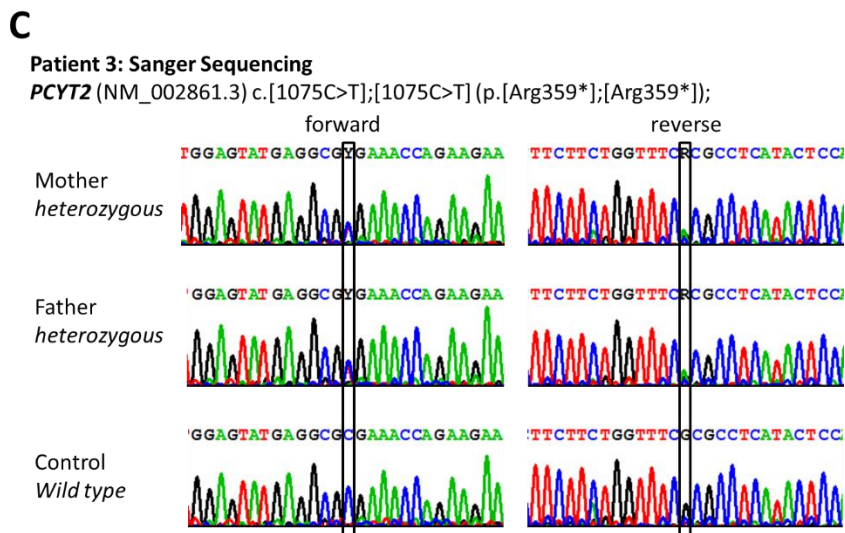
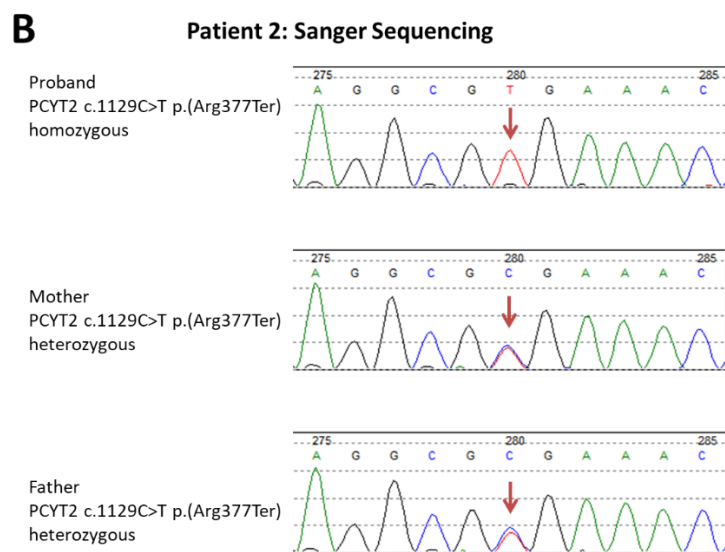
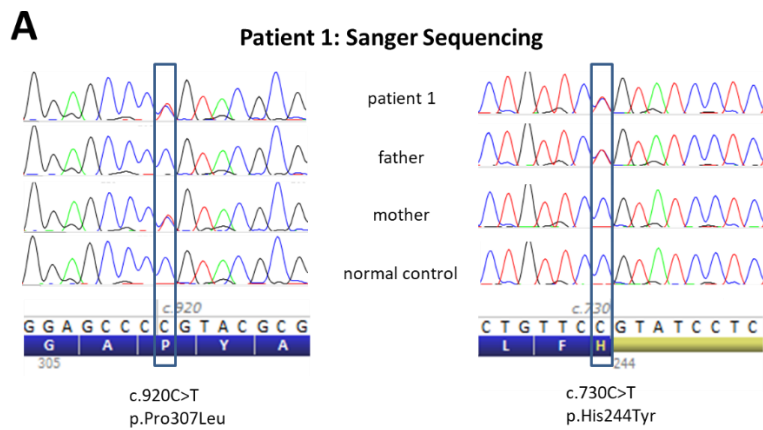
Supplementary figure 1: Zebrafish CRISPR efficiency. Restriction digest demonstrating CRISPR efficiency in day 5 zebrafish. The sgRNA were designed to target regions containing restriction enzyme sites. Efficiency of the CRISPR injections is indicated by loss of the restriction site, comparing embryos 1-5 against uncut wild type embryos and cut wild-type embryos. For guide sequences see Supplementary information 2.



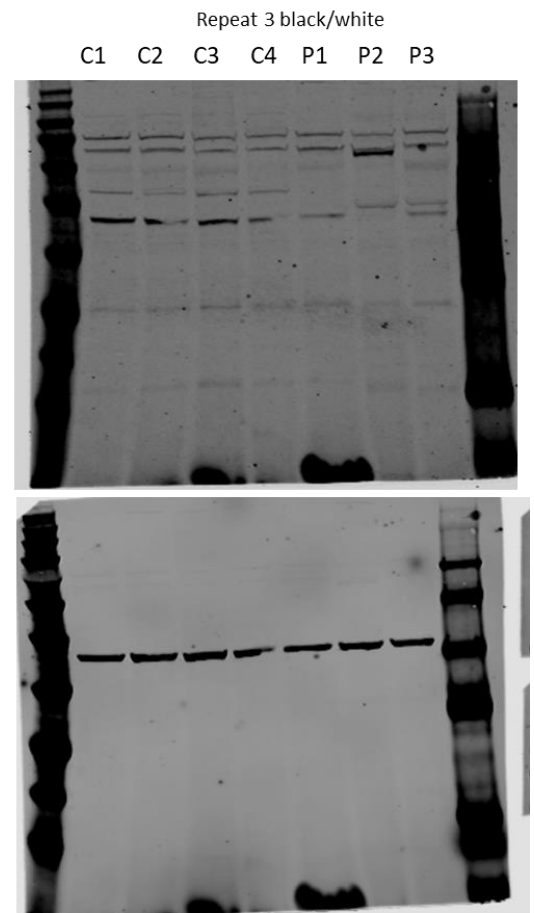
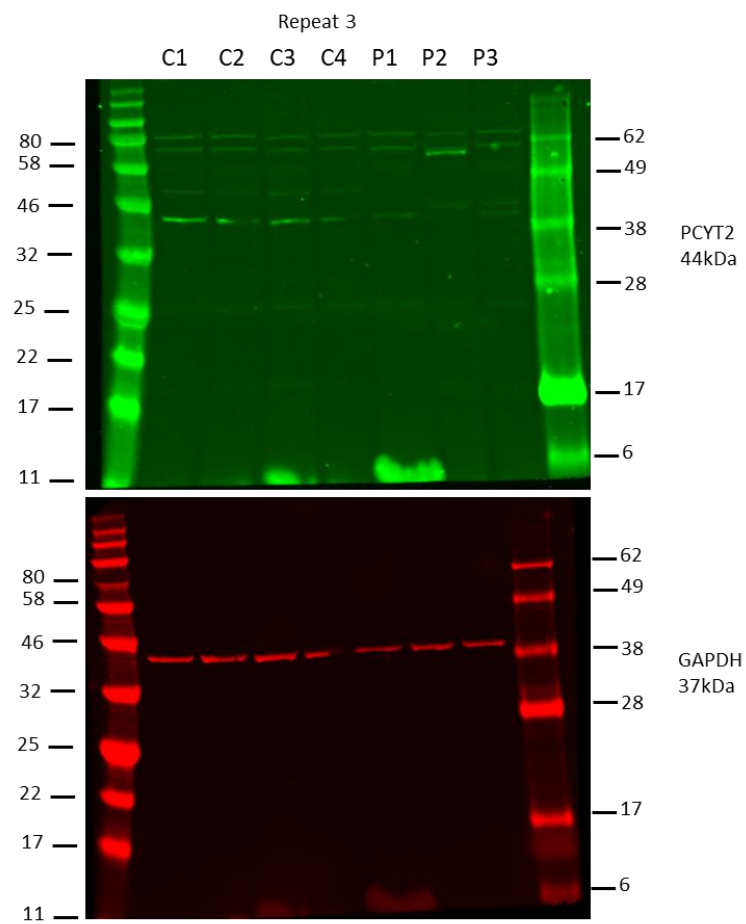
Supplementary figure 2: The efficiency of the CRISPR-CAS9 system was assessed by performing a restriction digest DNA extracted from tail-fin clippings. Good efficiency is indicated by a failure of the enzyme to produce a cut band, as this indicates that there has been complete loss of the restriction site caused by the CRISPR-CAS9 system. Poor efficiency meanwhile is demonstrated by complete cutting of the band, indicating that the restriction site is present. The mutant DNA was compared against an undigested and a digested wild-type zebrafish DNA. A) Only 3 pcyt2_03 model zebrafish survived to 6 weeks and genotyping revealed either a mosaic picture (1 & 3) or no cut at all (2). B) A sample of the pcyt2_13 models were genotyped which revealed a mosaic genotype in 5 out of the 6 samples (2-6).



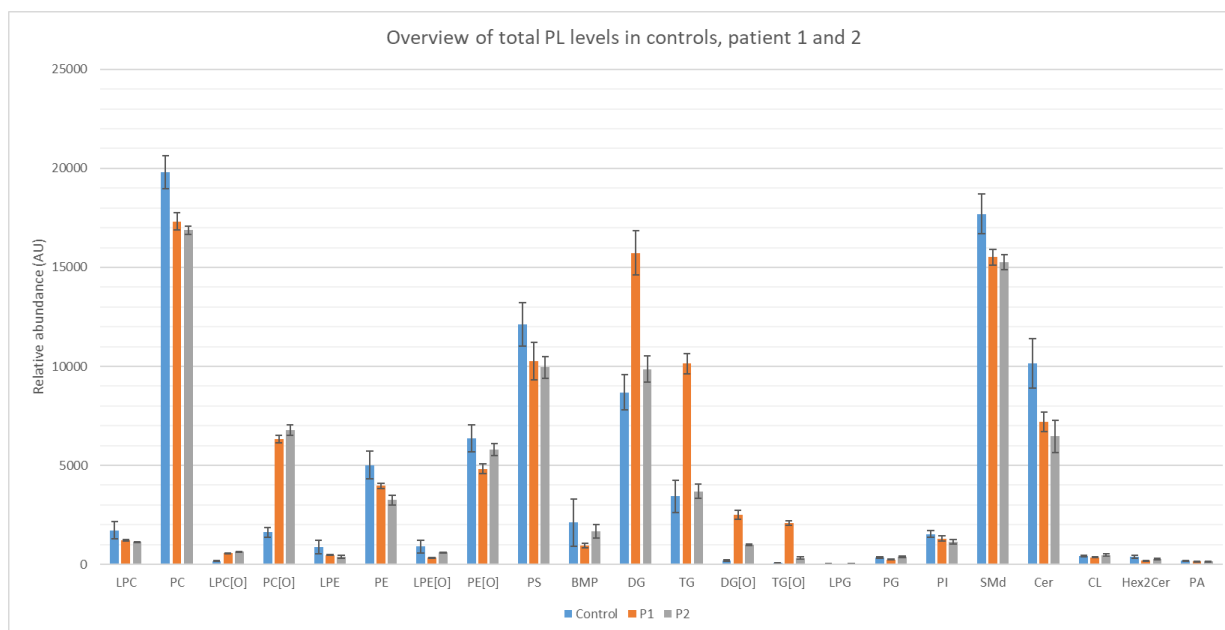
Supplementary figure 3: Transcript table detailing all known and predicted PCYT2 transcripts. PCYT2-203 represents the canonical transcript (NM_001184917) and the black arrow indicates exon 14, the region affected by the final exon nonsense variants in patients 2-5. Red transcripts indicate Ensembl protein coding transcripts. Yellow indicate protein coding transcripts predicted from Ensembl and Havana. Blue transcripts indicate non-protein coding transcripts. Figure adapted from Ensembl Genome Browser.



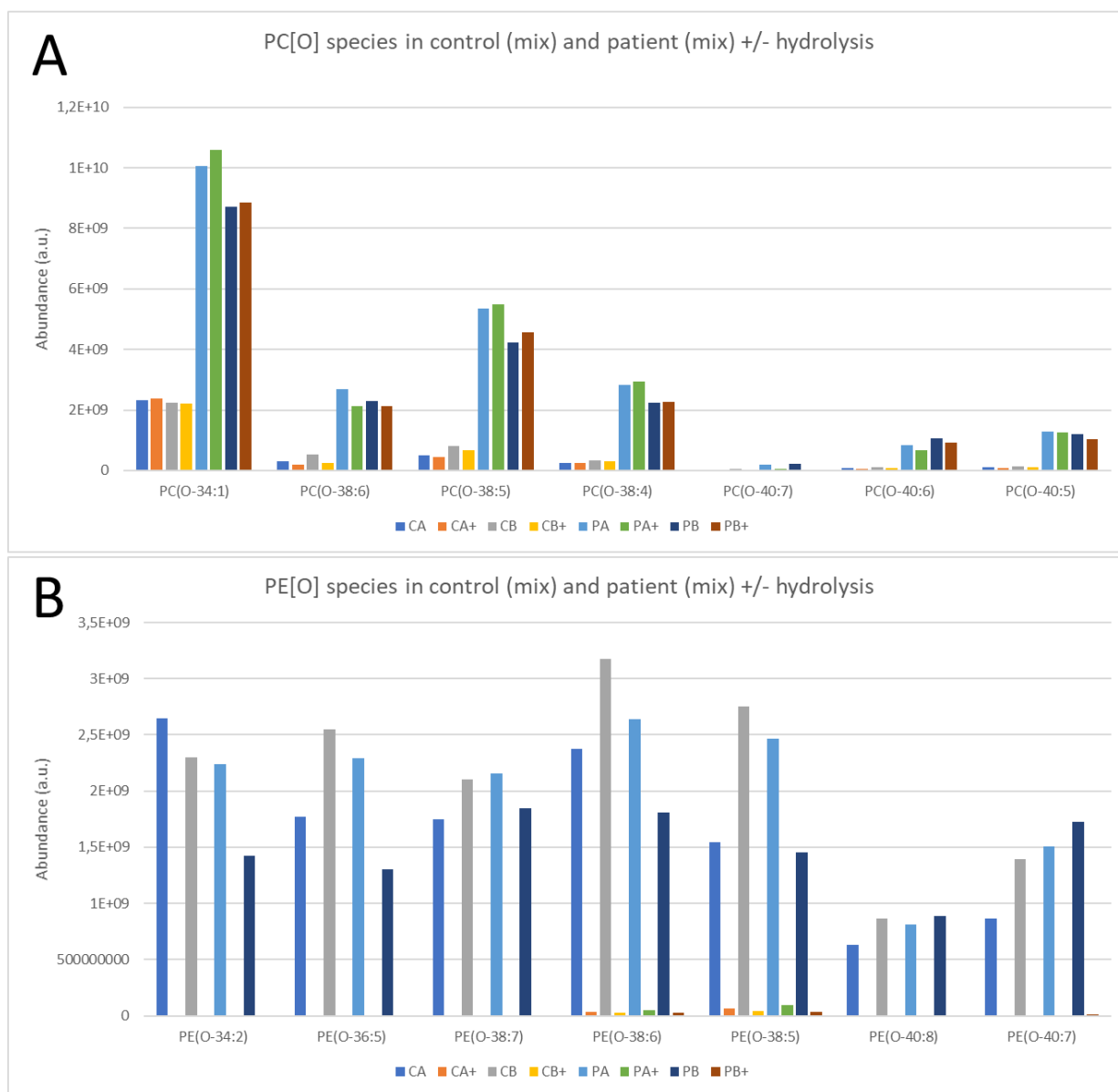
Supplementary figure 4: Sanger traces of variants for patients 1 (A) patient 1, (B) patient 2 and (C) patient 3.



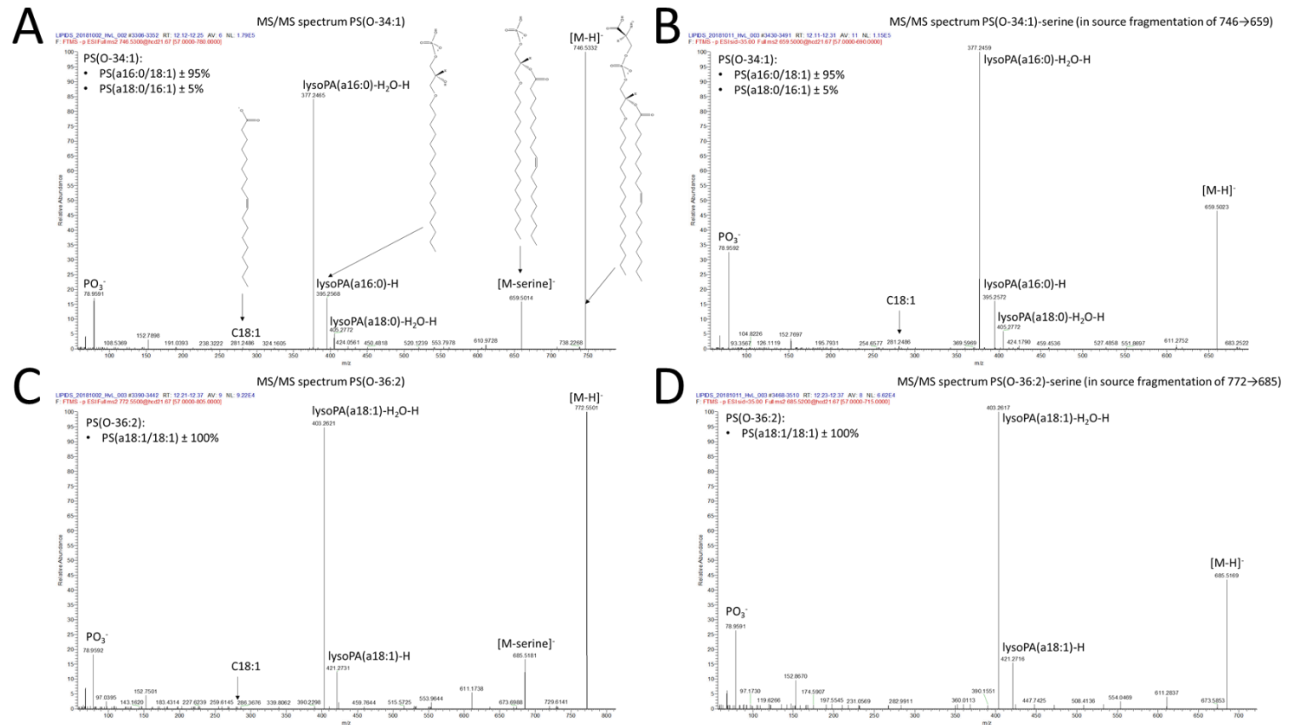
Supplementary figure 5: Complete Western Blot anti-PCYT2 and anti-GAPDH (false colour and black and white)



Supplementary figure 6: Overview of total phospholipid levels. Total phospholipid levels of controls, patient 1 and 2 (3 controls each measured as n=3), patient 1 (n=3) and 2 (n=4), mean \pm SD is shown) based on summed lipidomic species. Please note that comparison of amounts between different species is not possible due to the semiquantitative nature of the analysis. BMP, bis(monoacylglycerol)phosphate; Cer, Ceramides; CL, cardiolipin; DG diacylglycerol; DG[O], 1-alkyl-2-acylglycerol; HexCer, hexosylceramides; LPC, lysophosphatidylcholine; LPC[O], lysoplasm(a/e)nylcholine; LPE, lysophosphatidylethanolamine; LPE[O], lysoplasm(a/e)nylethanolamine; PA, phosphatidic acid; PC, phosphatidylcholine; PC[O], plasm(a/e)nylcholine; PE, phosphatidylethanolamine; PE[O], plasm(a/e)nylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; TG, triacylglycerol; TG[O], 1-alkyl-2,3-diacylglycerols.



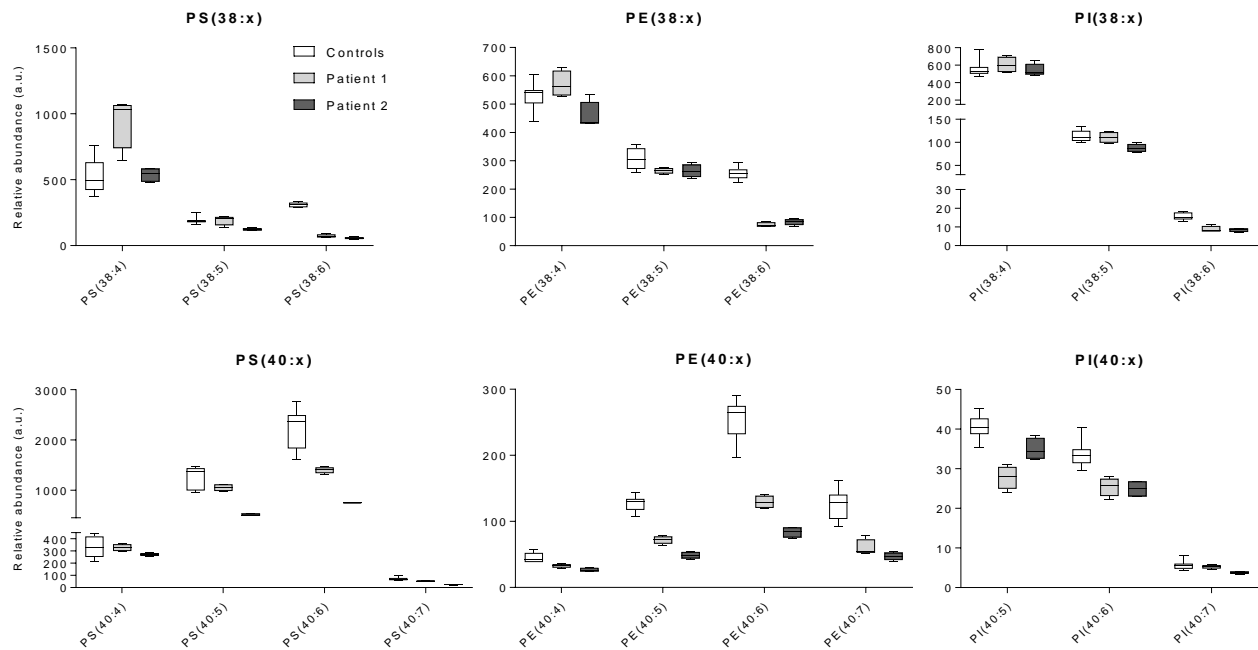
Supplementary figure 7: Plasmanyl/plasmenyl composition of selected etherphospholipid species. Plasmanyl/plasmenyl composition of (A) PC- and (B) PE-etherphospholipids: control and patient lipid extracts with and without treatment with 0.1 M HCl to hydrolyse plasmenyl species. PC-etherphospholipids are almost exclusively plasmanyl-species and PE-etherphospholipids are almost exclusively plasmenyl-species in both control and patient extracts.



Supplementary figure 8: Fragmentation confirms the occurrence of PS[O] in *PCYT2* patients.

(A) Fragmentation of m/z 746 (tentatively PS(O-34:1)) is dominated by the ions at m/z 659 (corresponding to loss of a serine head group), m/z 395 and 377 (by losses of 18:1-fatty acid substituent at sn-2 as an acid and as a ketene, respectively) and m/z 405 (loss of 16:1-fatty acid substituent at sn-2 as an acid). The spectrum also contains the ion at m/z 281, corresponding to a 18:1-carboxylate anion. The ions corresponding to the radyl substituent at sn-1 are not detected. The results show that the ion at m/z 746 is a mixture of different phosphatidylserines, this results in the structures PS(a16:0/18:1) and PS(a18:0/16:1) (where a=alkyl), with an abundance of about 95% and 5% respectively (based on the intensities of fragments m/z 377 and 405). (B) Fragmentation of m/z 659, an in source fragmentation product of m/z 746 (tentatively PS(O-34:1)) gives the same m/z 395, 377 and 405 fragments, confirming the identity of m/z 746 as PS(O-34:1). (C) Fragmentation of the ion at m/z 772 (tentatively PS(O-36:2)). This ion can be a plasmalyl-PS (a36:2) or plasmenyl-PS (p36:1). The fragmentation spectrum of m/z 772 is dominated by m/z 685 (corresponding to loss of a serine head group), m/z 403 and 421 (losses of 18:1-fatty acid substituent as an acid and as a ketene, respectively). The spectrum also contains the ion at m/z 281, corresponding to a 18:1-carboxylate anion. The results show that the ion at m/z 772 is a phosphatidylserine (PS) containing a 18:1-fatty acid substituent. (D) Fragmentation

of m/z 685, an in source fragmentation product of m/z 772 (tentatively PS(O-36:2)) gives the same m/z 403 and m/z 421 fragments. The absence of a fragment at m/z 279 (C18:2) and the ions corresponding to a radyl substituent at sn-1 supports the identification of PS(O-36:2) as the structure of PS(a36:2) = PS(a18:1/18:1) and not PS(p36:1) = PS(p18:0/18:1).



Supplementary figure 9: Levels of selected polyunsaturated phospholipid species. Box and whiskers plot of polyunsaturated species of PS, PE and PI. Species with 4 or more double bonds are shown for controls (white), patient 1 (light gray) and patient 2 (dark gray) and show a trend towards lower levels, especially for PE and PS.